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Dietary metformin effects on in vitro and in vivo metabolism in the chicken☆

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Abstract

Chickens were fed diets containing 0, 0.25, 0.5, and 1 and 0, 2.5, 5, and 10 g metformin (MET)/kg diet in 2 separate experiments to determine whether MET (1,1 dimethylbiguanidine hydrochloride) regulated plasma glucose and, possibly, feed intake in broiler chickens. Feed intakes in the first experiment were equal, but, in the second experiment, MET at 5 and 10 g/kg reduced feed intake. The first series of diets had no effect on plasma glucose and lactate. The second series of dietary treatments did not affect plasma glucose but did increase plasma lactate, uric acid, and triglycerides linearly. In the second experiment, there were significant decreases in lipogenesis that accompanied increasing doses of MET. The increase in plasma lactic acid suggests that MET stimulates pyruvate kinase in the chicken, as it does in mammals. The lack of effect on plasma glucose also suggests that regulation occurs downstream of pyruvate in the chicken. These findings may explain MET's ability to reduce hepatic triglyceride synthesis and suppress appetite.
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1. Introduction

There are many agents used to control blood glucose and insulin intolerance in diabetics. Some of these agents (biguanides) improve insulin sensitivity of diabetic tissue [1]. Insulin

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levels are low in chickens [2] with unknown metabolic effects. The relationship between plasma glucose and appetite is poorly understood in poultry. Exogenous hormone treatments have not resulted in marked changes in either feed intake or plasma glucose. Chickens are resistant to treatments that alter both plasma glucose and appetite in mammals. Recently, Ashwell and McMurtry [3] found that an acute dose of the biguanide metformin (MET) decreased food consumption as well as plasma glucose, insulin, and triglycerides. This observation supported some relationship between plasma glucose and food consumption by chickens.

As previously mentioned, a biguanide (MET) lowered plasma glucose levels in noninsulin-dependent human diabetics. The mechanism of action remains unknown, although, in some cases, reduction of hepatic glucose output may be the basis for MET's benefit in diabetes. The balance of the evidence suggests inhibition of gluconeogenesis [4-6]. Other sets of work indicate that biguanides reduce hyperglycemia by increasing insulin sensitivity, decreasing glucose absorption, and inhibiting hepatic gluconeogenesis. Studies have examined the effect of biguanides and MET in particular on hepatic glucose production and muscle glucose use and have yielded conflicting results and little information about the action of MET on lactate turnover and gluconeogenesis from lactate. Metformin has no effect on the rate of lactate turnover or gluconeogenesis from lactate [7]. In contrast, there is an increased flux through pyruvate kinase in MET-treated cells, suggesting allosteric activation of pyruvate kinase by fructose-1,6-diphosphate.

More recent work suggests that MET improves insulin-mediated glucose transport in isolated muscles. In contrast, in the absence of insulin no changes in basal glucose transport activity were observed [1]. These authors reported that part of the beneficial effect of MET on insulin resistance results from facilitating the hormone-stimulating effect on glucose transport in peripheral tissues (mainly skeletal muscle). In contrast to mammals, glucose production is the main method of regulating plasma glucose. The hypothesis tested in the experiments in this report was that chemically altering plasma glucose by inhibiting glucose production might decrease plasma glucose, change feed intake, and affect some indices of intermediary metabolism. In vitro lipogenesis (IVL) was measured in the chicken as an estimate of its capacity to synthesize fatty acids (FAs) de novo when fed a biguanide. Malic enzyme (ME) activity was monitored because it provides reducing equivalents (nicotinamide adenine dinucleotide phosphate [NADPH]) for the synthesis of FAs. Isocitrate: NADP+ oxidoreductase-[decarboxylating] (ICD) may both function as a residual source for the provision of NADPH and provide a coreactant for transamination. Aspartate aminotransferase (AAT) aids in the removal of excess amine groups formed during periods of protein degradation or inhibition of lipogenesis.

2. Methods and materials

2.1. Animals and diets

We formulated diets that were slightly low in crude protein (18%) containing 0, 0.25, 0.5, and 1 g of MET per kilogram (experiment 1). Chickens were placed on these diets at 7 days of age and allowed to grow for 21 days. In the second experiment, we followed the above

Table 1
Effects of MET on in vitro metabolism and plasma metabolites in broiler chickens (experiment 1)[a]

MET[b]	FA[c]	ME[d]	ICD[d]	AAT[d]	Plasma[e] glucose	Plasma[e] lactate
0	43.4 ± 3.6	20.7 ± 0.8	26.5 ± 2.1	52.6 ± 2.5	260 ± 4.2	17 ± 3.4
0.25	43.4 ± 3.9	20.1 ± 1.4	23.0 ± 1.2	46.9 ± 2.1	255 ± 3.8	21 ± 3.8
0.5	34.8 ± 2.5	19.4 ± 0.8	23.9 ± 1.4	50.8 ± 2.6	242 ± 8.5	28 ± 3.1
1.0	27.1 ± 1.9	20.3 ± 1.2	25.5 ± 1.2	53.4 ± 2.9	257 ± 5.2	29 ± 3.8
MET	$P < .05$	NS	NS	NS	NS	NS

[a] Broiler chickens were grown under standard conditions from 0 to 7 days of age and then fed diets containing these levels of MET from 7 to 28 days of age.
[b] Grams MET per kilogram diet.
[c] FA synthesis; noted as micromoles of [^{14}C] acetate incorporated per gram of liver into FAs.
[d] All activities are expressed as micromoles of oxidized or reduced NAD(P) produced per minute per gram of liver.
[e] Milligrams per 100 mL of plasma.

protocol but we fed diets containing 0, 2.5, 5, and 10 g MET per kilogram. In both experiments, 28-day-old chickens were bled and then killed by cervical decapitation. All chickens were held under a quarantine that was certified by the station veterinarian and were observed daily for healthiness. One authorized animal caretaker was assigned to maintain chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Center Institutional Animal Care Committee. Plasma was stored at −80 °C until analyzed for metabolites. Livers were washed in 155 mmol/L NaCl and then either sliced (MacIlwain Tissue Chopper, Goomshall, UK; 0.4-0.5 mm) or rapidly frozen in liquid N_2 and then stored at −80 °C.

2.2. In vitro metabolism

Quadruplicate liver explants were incubated at 37 °C for 2 hours in Hanks' salts [8,9] containing 10 mmol/L HEPES and 10 mmol/L sodium [2-^{14}C]acetate (166 MBq/mol). Incubations were in 3-mL volumes at 37 °C for 2 hours under a 95% O_2–5% CO_2 atmosphere. The explants were then placed in 10 mL of 2:1 chloroform/methanol for 18 hours according to Folch et al [10]. In vitro lipogenesis was expressed as micromoles of acetate incorporated into hepatic lipids per kilogram of body weight.

Samples of the frozen liver tissues were homogenized (1:10, wt/vol) in 100 mmol/L HEPES (pH 7.5)–3.3 mmol/L 8-mercaptoethanol and centrifuged at 12 000 × g for 30 minutes [8,9]. The supernatant fractions were kept at −80 °C until analyzed for certain enzyme activities. Malic enzyme activity (EC 1.1.1.40) was determined by the method of Hsu and Lardy [11]. Isocitrate: NADP+ oxidoreductase-[decarboxylating] (EC 1.1.1.42) activity was determined by the method of Cleland et al [12]. Aspartate aminotransferase (EC 2.6.1.1) activity was determined by the method of Martin and Herbein [13].

2.3. Statistical analysis

Significance of overall treatment effects was determined with analyses of variance with MET being the main treatment effect in both experiments. The procedures of statistical analyses have been previously described [8,9].

Table 2
Effects of MET on in vitro metabolism and plasma metabolites in broiler chickens (experiment 2)[a]

MET[b]	Glucose[c]	Lactate[c]	UA[c]	NEFA[d]	Cholesterol[c]	FA[e]	ME[f]	ICD[f]	AAT[f]
0	251 ± 2.9	20.2 ± 2.1	3.8 ± 0.2	244 ± 17.2	129 ± 2.9	35.4 ± 3.9	19.2 ± 1.2	28.6 ± 1.2	57.4 ± 3.3
2.5	259 ± 3.8	37.5 ± 4.2	4.5 ± 0.2	213 ± 9.5	150 ± 5.8	30.2 ± 2.2	20.3 ± 1.5	30.4 ± 1.3	61.3 ± 3.8
5.0	254 ± 3.5	28.5 ± 1.6	5.0 ± 0.6	319 ± 17.6	149 ± 6.4	26.7 ± 3.2	16.6 ± 1.8	36.4 ± 1.9	71.9 ± 3.4
10.0	253 ± 2.9	64.3 ± 5.1	5.1 ± 0.2	291 ± 21.4	145 ± 12.8	22.9 ± 2.2	13.6 ± 1.5	46.7 ± 2.3	78.1 ± 5.4
MET	NS	$P < .05$	$P < .05$	NS	NS	$P < .05$	$P < .05$	$P < .05$	$P < .05$

[a] Broiler chickens were grown under standard conditions from 0 to 7 days of age and then fed diets containing these levels of MET from 7 to 28 days of age.
[b] Grams MET per kilogram diet.
[c] Milligrams per 100 mL of plasma.
[d] Micro-equivalents per liter.
[e] FA synthesis; noted as micromoles of [^{14}C]acetate incorporated per gram of liver into FAs.
[f] All activities are expressed as micromoles of oxidized or reduced NAD(P) produced per minute per gram of liver.

3. Results

Table 1 summarizes the effects of the low range of MET supplementation on some indices of intermediary metabolism. The significant effect of MET ($P < .05$; FA) on IVL was due to the 2 higher doses of MET (0.5 and 1.0 g/kg) as the lowest dose (0.25 g/kg) resulted in a rate similar to the control diet. In contrast, ME, ICD, and AAT were unaffected by MET. Metformin did not affect plasma glucose in 28-day-old chickens. In contrast, the 2 higher doses of MET (0.5 and 1 g/kg diet) increased ($P < .05$) plasma lactate compared to the control, unsupplemented diet. Body weights and feed intakes were generally unaffected by MET at these levels and are not shown.

Table 2 summarizes the effects of higher doses of MET on some indices of in vitro metabolism. Significant negative linear effects of MET ($P < .05$) were noted for both FA synthesis and ME activity. In contrast, significant positive effects were observed for both ICD and AAT activities. Table 2 indicates that chronic MET feeding had no effect on plasma glucose, free FAs (nonesterified FA), or cholesterol. In contrast, both plasma lactate and uric acid (UA) were increased ($P < .05$) by dietary MET.

4. Discussion

The results of these experiments show that plasma glucose is resistant to drugs that remarkably decrease mammalian glucose. We are aware of only a few studies showing an actual change in avian blood glucose caused by some pharmacological agent. Chida et al [14] have reported that hepatic glycolysis increases slightly in persistent hypoglycemic chickens. Simon et al [15] demonstrated that plasma glucose level was not altered by a glucagon analog but was increased by insulin-immune serum. Simon et al [15] found that hyperglycemia elicited by insulin antisera had no effect on food intake.

The data in this report tend to support a previous hypothesis that MET operates through a protein kinase to inhibit lipogenesis [3]. We [8,9,16] have reported extensively on the regulation of catechol hormones and cAMP which function by protein phosphorylation. Briefly, incubation of liver explants in the presence of isoproterenol or dibutyl cAMP decreased IVL, regardless of the background nutritional or hormonal status of the chicken studied.

Ashwell and McMurtry [3] found that a single dose of MET decreased plasma glucose, insulin, and triglycerides 3 hours postdosing. Likewise, this dose decreased feed intake for up to 24 hours postdosing. It is evident to us that, at least in the chicken, an acute MET treatment must elicit a change in metabolism to affect feed intake. Mere temporal changes in plasma glucose may not be the sole reason for observed changes in feed intake. The present study indicates that feeding very high levels of MET decreased feed intake without affecting plasma glucose. It is evident that methods of administering MET will give radically different results with the chicken. It should be noted, however, that either method of MET administration (inclusion in feed or by gavage) will increase plasma lactate concentrations.

Perhaps, a few comparisons with mammals might explain reasons why biguanides do not affect avian plasma glucose as markedly as mammalian plasma glucose. Santure et al [1] reported that an improvement in insulin-mediated glucose transport activity was detected in isolated muscles from MET-treated hypertensive rats, but in the absence of insulin, no

changes in basal glucose transport activity were observed. It is further suggested that part of the beneficial effect of MET on insulin resistance results from a potentiation of the hormone-stimulating effect on glucose transport in peripheral tissues (mainly skeletal muscle). Although it may be difficult to compare results from isolated tissue preparations to the whole animal, it is tempting to speculate that at least part of our findings could be explained by low plasma insulin levels in chickens [2]. Particularly relevant is the study by Landin et al [17], in which MET treatment of nonobese, nondiabetic untreated hypertensives led to an improvement in insulin sensitivity and reduction in blood pressure that was reversed after cessation of treatment. Similar results were found in obese, hypertensive women [18] and obese, type II diabetics in which the reduction of blood pressure was related to the antihyperglycemic effect [19].

In conclusion, dietary MET administration did not change plasma glucose of chickens. This effect may be specific to chronic dosing that introduces metformin to intermediary metabolism at a fixed rate. In contrast, MET did increase plasma lactate which might indicate a change in the flux rate of metabolites through glycolysis, altering feed intake by some sort of feed back inhibition. The decrease in lipogenesis accompanying dietary MET supports restriction in lipogenic substrates.

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